International patent application No. PCT/DK97/00342

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Artificial promoter libraries for selected organisms and promoters derived from such libraries

Our ref: 20911 PC 1

NEW CLAIMS, 26 NOVEMBER 1998

1. A promoter library suitable for optimising the expression of a gene in a selected organism or group of organisms, said library comprising a set of different individual promoter sequences covering, with respect to promoter strength for said gene, a range of promoter activities, the set of different individual promoter sequences comprising double stranded DNA sequences, the sense strands of which comprise

at least two consensus sequences of a promoter sequence identified in said organism or group of organisms, at least half of each of said consensus sequences is kept constant in all of the individual promoter sequences and, between said consensus sequences or flanking at least one of said consensus sequences, a nucleotide spacer sequence, at least part of which, relative to the corresponding spacer sequence of the identified promoter, is varied to comprise nucleotides that are selected randomly among the nucleobases A, T, C and G,

the promoter library spanning, with respect to promoter activitities for said gene, a range of interest, in small steps, each step preferably changing the activity by 50-100%.

2. A promoter library according to claim 1 wherein at least 10 nucleotides in the spacer sequence(s) are selected randomly among the nucleobases A, T, C and G.

3. A promoter library according to claim 1 wherein the promoter sequences comprise a regulatory DNA sequence imparting a specific regulatory feature to the promoters of said promoter sequences.

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4. A promoter library according to claim 1 wherein the promoter sequences comprise at least one recognition site for restriction endonuclease

5. A promoter library according to claim 1 wherein the selected organism or group of organisms is selected from prokaryotic organisms.

6. A promoter library according to claim 5 wherein the consensus sequences comprise at least 3 conserved nucleotides of the –10 signal TATAAT.

A 7. A promoter library according to claim 5 ~~or 6~~ wherein the consensus sequences comprise at least 3 conserved nucleotides of the –35 signal TTGACA.

8. A promoter library according to claim 5 wherein the consensus sequences further comprise intervening conserved motifs.

A 9. A promoter library according to ~~any of claims 5-8~~ claim 5 comprising at least two promoter sequences selected from the group consisting of SEQ ID NO:5 to SEQ ID NO:42

10. A promoter library according to claim 7 wherein the spacer sequence between the –35 and the –10 signal is 14-23 bp.

11. A promoter library according to claim 5 wherein the promoter sequences comprise a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2 and minor variations hereof.

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12. A promoter library according to claim 1 wherein the selected organism or group of organisms is selected from eukaryotic organisms.

13. A promoter library according to claim 12 wherein the consensus sequences comprise a TATA box and at least one upstream activation sequence (UAS).

A 14. A promoter library according to claim 12 ~~or 13~~ wherein the promoter sequence is selected from the group consisting of SEQ ID NO:3 and minor variations hereof.

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claim 12

15. A promoter library according to ~~any of claims 12-14~~ comprising at least two promoter sequences selected from the group consisting of SEQ ID NO:43 to SEQ ID NO:58

16. A method of constructing a set of promoters (a promoter library) which is suitable for optimising the expression of a gene in a selected organism or group of organisms, the method comprising the steps of

(i) identifying in said organism or group of organisms a promoter sequence comprising at least two consensus sequences separated by a non-conserved nucleotide sequence (a spacer sequence),

(ii) constructing a set of single stranded DNA sequences comprising at least half of each of the consensus sequences of the identified promoter sequence, and a non-conserved nucleotide spacer sequence, at least part of which, relative to the spacer sequence of the identified promoter, is varied to comprise nucleotides that are selected randomly among A, T, C and G, whilst keeping the at least half of the consensus sequences constant, and

(iii) converting the single stranded DNA sequences into double stranded DNA sequences

to obtain a set of different promoters covering, with respect to promoter strength, a range of promoter activities.

17. A method according to claim 16 wherein the set of different promoters obtained is a promoter library according to any of claims 1-15.

18. A method of optimising the expression of a gene in an organism, the method comprising

(i) selecting from the promoter library of ~~any of claims 1-15~~ claim 1 a set of promoters covering a desired range of promoter activities,

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(ii) cloning said set of promoters into the organism placing in each clone the gene to be expressed under the control of at least one promoter of the set,

(iii) cultivating the clones and selecting a clone showing optimised flux of gene product formation.

19. A method according to claim 18 wherein the increase in activity from one promoter to an other promoter of the set of promoters is in steps that do not exceed 50-100%.

20. A method according to claim 18 wherein the selected organism is selected from the group consisting of a prokaryotic organism and a eukaryotic organism.

21. A method of isolating a promoter sequence being capable of optimizing the expresssion of a gene in a selected organism, the method comprising

(i) constructing, using the method of claim 16 ~~or 17,~~ a set of promoters covering, with respect to promoter strength, a range of promoter activities,

(ii) cloning said set of promoters into the selected organism placing in each clone the gene to be expressed under the control of at least one promoter of the set,

(iii) cultivating the clones and selecting the clone showing optimized flux of gene product formation, and

(iv) isolating said promoter sequence from the clone showing optimized flux of gene product formation.

22. A promoter sequence that is capable of optimising the expression of a gene in a selected organism, the promoter sequence is obtainable by the method of claim 21.